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INHIBITION OF NEMATOCYST DISCHARGE DURING FEEDING IN THE COLONIAL HYDROID *HALOCORDYLE DISTICHA* (= *PENNARIA TIARELLA*): THE ROLE OF PREVIOUS PREY-KILLING

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Abstract

The colonial hydroid *Halocordyle disticha* (= *Pennaria tiarella*) kills prey with stenotele nematocysts. These nematocysts become less responsive after heavy feeding, but their killing activity is restored after hydranths are placed in fresh medium. Nematocysts of unfed hydranths became inactive when neighboring hydranths (either attached or separated) were fed; the effect increased with the amount of previous prey-killing. The loss of nematocyst activity was not due to stenotele depletion or compounds in prey fluids; waste products produced by the hydroid or prey had little effect. Extracts of capitate tentacles produced inactivation, and stenoteles were completely inhibited in a concentrated solution of stenotele discharge products. Our results indicate that the inactivation of stenoteles is due to the accumulation of materials released from the stenoteles during discharge.

Introduction

Very little is understood about the mechanisms which control nematocyst discharge in cnidarians. These intracellular secretion products ("organoids") classically have been considered to be independent effectors, acting as both receptor and effector without input from conducting pathways. More recent studies indicate some control over nematocyst discharge, as it may be influenced by the physiological state of the animal (reviewed in Mariscal, 1974). There is ample evidence for neuro-nematocyst junctions (Westfall, 1969; Westfall *et al.*, 1971), and non-neural epithelial conduction (Mackie, 1970) may also function in controlling discharge.

Repeated observations that satiated animals fail to capture or even reject offered food (Hyman, 1940; Kanaev, 1952; Mariscal, 1974) provide evidence for host control over nematocyst function. These studies have been performed extensively with *Hydra*. Burnett *et al.* (1960) suggested that the stimulation of gastrovascular stretch receptors in well-fed animals mediated stenotele inactivation, while Smith *et al.* (1974) presented evidence that perception of prey metabolites by gut cells was the sensory clue for nematocyst inhibition. More recently Ruch and Cook (1984) demonstrated that inactivation following feeding could occur without food in the gut, and that soluble factors released by the hydra during feeding served to inactivate stenoteles. The latter conclusion was based on the finding that feeding by a parent hydranth reduced the stenotele activity of nearby buds which had no gut connections with the parents, even when those buds had recently detached.

This observation raises the question of nematocyst inactivation during feeding

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by colonial cnidarians. We chose the colonial athecate hydroid *Halocordyle disticha* (= *Pennaria tiarella;* Calder and Hester, 1978) for study, as it is readily available and much is known about its feeding biology (Pardy and Lenhoff, 1968). Our research was designed to investigate three questions: (1) does stenotele inactivation occur during feeding by this hydroid? (2) How does feeding by one hydranth affect nematocyst function in neighboring hydranths? (3) What is the source of any inactivating factors?

Materials and Methods

Colonies of *Halocordyle disticha* (= *Pennaria tiarella*) were collected from Coney Island, Bermuda, or Morehead City, North Carolina. They were maintained in running seawater at 23–27°C in nine-inch culture dishes, and fed *Artemia salina* nauplii twice daily until the day prior to use in experiments. At this time they were placed in dishes containing aerated, filtered (0.45 μm) seawater. For feeding experiments, groups of three hydranths from the same colony (either connected by the stolon or separated) were placed in small plastic dishes with 0.15 ml of seawater. In one experiment the volume was increased to 150 ml to study the influence of the volume of fluid on nematocyst activity.

Feeding procedure and assay of killing response

All responses of *H. disticha* were observed under a dissecting microscope at 30×; in some experiments a video camera fitted to a Wild M420 Macroscope was used to record observations. We used two methods to quantitatively present *Artemia* larvae to the hydranths. In the first, "planned random feeding," nauplii were drawn into a capillary tube (I. D. 0.9 mm) fitted to a mouth pipette and the total counted under a dissecting microscope. These were fed to the hydranths, and additional *Artemia* were added if all of the original allotment had been killed. Any nauplii which were still alive after inactivation of all three hydranths (see below) were subtracted from the total presented to yield the number which had been killed. In the second method, "selective feeding," nauplii were individually presented to hydranths with the tip of a flame-drawn capillary tube. Care was taken to avoid letting any prey escape and that only particular hydranths were fed at any one time.

In both procedures, we considered the nematocysts of a hydranth to be inactive when five consecutive contacts with larvae produced neither killing nor capture (adherence to tentacles; Ruch and Cook, 1984). Typically the time required to feed control hydranths to inactivation was less than 40 minutes.

Selective feeding of connected and separated hydranths

To determine if feeding by neighboring hydranths affected the nematocyst activity of a hydranth, we performed experiments involving hydranths connected by a stolon, and hydranths which had been separated by cutting the stolon. The separated hydranths were allowed 6 h to heal and then were placed 2 mm apart in a dish; this spacing corresponded to their usual positions in an intact colony. In each case successive polyps were "selectively" fed to inactivation. After inactivation the connected hydranths were separated, and all of the hydranths transferred to individual dishes with fresh filtered seawater. They were then tested for the recovery of feeding ability.

Determination of stenotele complements in fed and unfed hydranths

Stenotele numbers in 24-hour starved hydranths and those which had been fed until nematocysts no longer functioned were compared in cell maceration prepara-

tions (David, 1973). Individual hydranths were separated, and the number of large (6.8 μm) stenoteles in capitate tentacles were counted using phase contrast microscopy (400×).

Media exchange experiments

To determine if factors released from either prey or from hydranths were effective in producing nematocyst inactivation, we determined the killing responses of hydranths which were fed in solutions in which other hydranths had fed. Each original group of three hydranths was fed to inactivation by the "planned random feeding" technique. The solution was drawn off with a fine-tipped micropipette, free of dead *Artemia* or other debris, and placed in a clean container. A fresh branch with three hydranths was then placed in this "used" solution and the hydranths were fed in the same manner. The number of *Artemia* killed at inactivation was then compared in both groups.

The effect of prey homogenate on stenotele activity

In an attempt to determine if prey fluids could be the source of any inactivating factor, hydranths were fed in a prey homogenate solution. Two hundred *Artemia* nauplii were homogenized in 0.5 ml filtered seawater with a hand grinder. To maximize cell disruption, the solution was electrified with platinum electrodes attached to a Grass S6 stimulator set at 80 V for 3 s. This solution was then filtered (0.45 μm), and a branch with three hydranths was fed by "planned random feeding" in 0.15 ml of the filtrate. Each test solution thus contained the extract of approximately 70 nauplii. Control hydranths were fed in electrified filtered seawater.

Feeding in capitate tentacle solution

We investigated the possibility that capitate tentacles were the source of inactivating factors by feeding hydranths in extracts of capitate tentacles. Two hundred capitate tentacles were homogenized in 0.5 ml of filtered seawater; each 0.15 ml feeding volume thus contained the extract of approximately 60 tentacles. The homogenate was electrified as above to induce stenotele discharge (see Results), and the resultant solution was filtered. As a control the internodal tissue was extracted from five 4-cm sections of stolon. This tissue, which is not involved with prey-killing, was treated in the same manner as the tentacles. Groups of three connected hydranths were then fed by "planned random feeding" in each solution.

Feeding in purified nematocyst solution

"Planned random feeding" of hydranths also occurred in an extract of purified nematocysts. Nematocysts were obtained from *H. disticha* using a modification (P. Suchy, in prep.) of the technique of Lane and Dodge (1959). Perisarc-free hydranths were gently stirred with a magnetic stirrer in 10 volumes of distilled water for 24 h at 4°C to autolyse tissue. The resulting suspension was screened through bolting silk and 35 μm and 28 μm Nytex screening to remove large particulates and centrifuged at high speed with an IEC benchtop centrifuge. The sedimented material was resuspended twice in 10 m*M* MOPS adjusted to pH 7.4 with Trizma base and centrifuged at high speed for 5 min. After this procedure, microscopic examination revealed that 97% of the particulate material consisted of nematocysts, the rest being unidentifiable

cellular debris. These preparations were stored frozen with the addition of 30 μg/ml of aprotinin (Sigma) as a protease inhibitor.

The final concentration of nematocysts was determined with a hemocytometer to be 85,000 per ml. The thawed solution was centrifuged at high speed in an IEC clinical centrifuge for 2 min, and the nematocysts were resuspended in filtered seawater. The solution was then electrified at 80 V for 3 s to induce stenotele discharge, and the solution passed through a 0.45 μm filter to remove particulate material. Thus the volume used in our feeding experiments (0.15 ml) contained the discharge products of approximately 12,000 stenoteles (20 times the stenotele complement of a single hydranth; Table I). Three connected hydranths were placed in this solution, while the control group was placed in electrified filtered seawater. Both groups were then tested by "planned random feeding."

Results

The effect of feeding by neighboring hydranths on nematocyst function

We studied the effect of feeding by neighboring hydranths on prey-killing by selectively feeding each of three connected hydranths until prey-killing ceased. The results of feeding the terminal hydranth first, followed by feeding the middle and then the innermost hydranth, are shown in Figure 1a. The middle hydranths showed a 46.6% decrease in activity relative to the first-fed hydranth, while those which were fed last showed an 83.8% decrease. The correlation between the number of nauplii killed and the order of feeding was highly significant ($r = -0.967$, $P < 0.01$; $n = 18$), showing clearly that feeding by hydranths reduced the nematocyst activity of neighboring polyps, and that the effect appears to be cumulative.

In other experiments we varied the order by feeding first the middle hydranth with virtually identical results (Fig. 1b). The hydranths which were fed second showed a decrease of 51.2%, while those which were fed last showed an 84.8% decrease. The correlation was the same in as the previous experiment ($r = -0.967$, $P < 0.01$; $n = 9$).

To determine if inactivation was due to information passed between the hydranths by neural elements, by other cell-cell contacts, or via the gastrovascular cavity we repeated the experiment with groups of three hydranths which had been dissected apart. Again, there was a decrease in nematocyst function with increased feeding. Relative to the initial value, the killing response of the hydranth which was fed second decreased by 58.6%, while the hydranth which fed last showed a 92.1% decrease (Fig. 2). The correlation between the killing response and the order of feeding was again highly significant ($r = -0.944$, $P < 0.01$; $n = 9$). This result demonstrates that decreased killing occurs without cellular interactions between polyps. Clearly, neither nervous inputs from prior fed hydranths nor food ingested by these hydranths are required for stenotele/cnidocyte inactivation by affected hydranths.

Figure 3 summarizes the effect of prior feeding on the killing response. This figure contains the combined data (connected and separated hydranths) for the second- and third-fed hydranths of Figures 1 and 2. The percentage of inhibition of killing (relative to the first-fed hydranth) is plotted against the number of shrimp which were killed in the solution prior to testing. The significant negative correlation ($r = -0.694$, $P < 0.01$) demonstrates that prior feeding by hydranths of *H. disticha* reduces the killing response of neighboring polyps, and indicates that the effect is additive, possibly due to the accumulation of factors in the feeding environment.

"Recovery" experiments confirmed the role of accumulating factors in stenotele

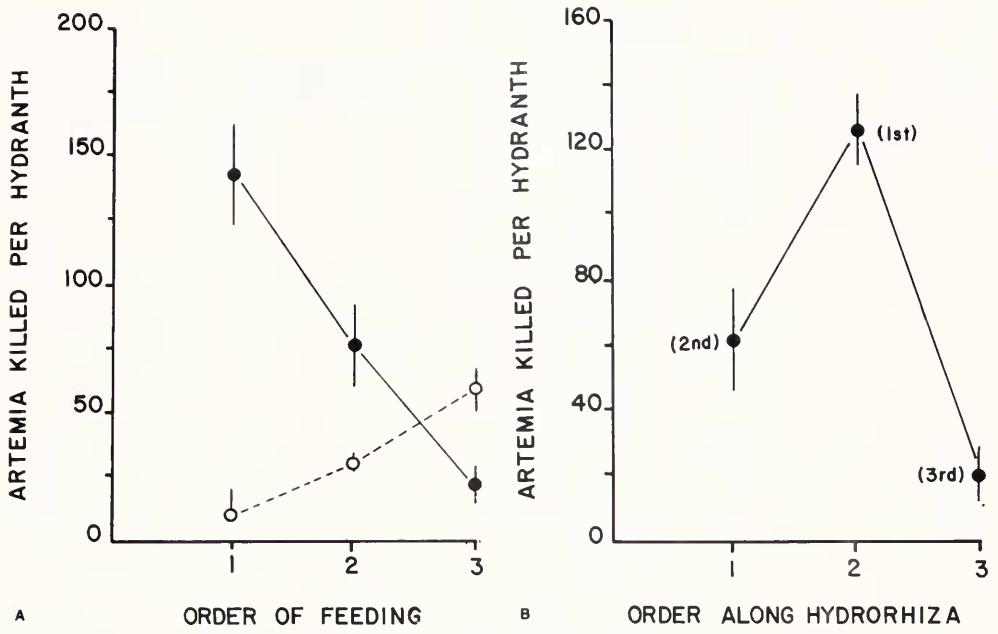

FIGURE 1. Stenotele activity during selective feeding of connected neighboring hydranths of *H. disticha*. Vertical bars = ±1 standard deviation. A. Selective feeding of hydranths in order along branch. ●——●, stenotele inactivation during feeding; ○---○, recovery following transfer to fresh medium. n = 6 for each point; r = −0.967. Variation in feeding order: middle hydranth fed first, then left, then right; order of feeding is indicated. n = 3 for each point.

inhibition. Branches of three connected hydranths were fed to inactivation, then rinsed in filtered seawater and dissected apart. The separated hydranths were then retested for their killing ability in individual dishes. The hydranths which had been fed first, and which originally had killed the most nauplii killed an additional 10.8 ± 10.0 shrimp (n = 3). The middle hydranths showed an intermediate killing response ($\bar{X}$ = 30.7), while the last-fed hydranths (which had killed very few *Artemia*) killed an average of 59.3 ± 7.5 shrimp. Thus, all three groups showed a reversal of the inactivation upon return to fresh seawater, consistent with the accumulation of inactivating factors in the original feeding solution. However, neither the second nor the third hydranths in each series killed as many total *Artemia* as the original number killed by first-fed hydranths.

Stenotele complements of fed and unfed hydranths

The effects of feeding neighboring polyps on nematocyst function indicated that the loss of nematocyst function with feeding accrued from the inactivation of nematocysts, rather than their depletion during feeding. To examine this possibility, we counted stenoteles in the capitate tentacles of groups of three connected hydranths, fed selectively as above. Twenty-four-hour starved hydranths served as controls.

The stenoteles of capitate tentacles could be readily distinguished from those of the filiform tentacles by their location and greater size (6.8 μm *vs.* 4.5 μm). We used these because they were more conspicuous, and were conveniently fewer in number. We did not distinguish between discharged and undischarged stenoteles in our

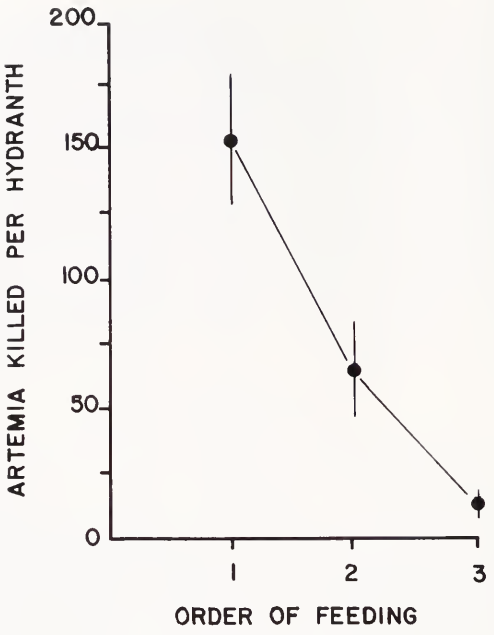

FIGURE 2. Inactivation of stenoteles during feeding of separated hydranths of *H. disticha*. Each hydranth selectively fed to inactivation before the next hydranth was tested. Order of feeding as in Figure 1a. n = 3 for each point; r = −0.944.

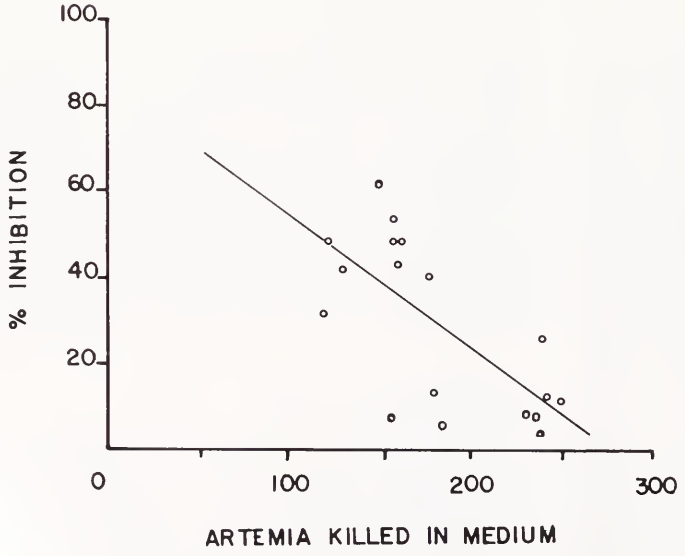

FIGURE 3. The effects of prior feeding on the killing responses of the second- and third-fed hydranths. The inhibition of killing is expressed as the percentage of *Artemia* nauplii killed by first-fed hydranths. The regression line is significant at the 1% level. (Data of Figs. 1 and 2.)

TABLE I

Complements of large (6.8 μm) stenoteles in fed and unfed hydranths of Halocordyle disticha *determined from counts of cell maceration preparations*

Feeding regime	Stenoteles per hydranth ($\bar{X} \pm$ S.D.)
First-fed	98 ± 20.5 ($P < 0.001$)*
Second-fed	283 ± 40.3 ($P < 0.001$)*
Third-fed	706 ± 45.1 (n. s.; $P > 0.3$)*
24 h starved	737 ± 29.1

* Probability that mean is equal to control value; one-way ANOVA.
The sequence of feeding of neighboring hydranths corresponds to that in Figure 1.
n = 3 for all cases.

counts. Unfed hydranths possessed 737 ± 29.1 (n = 3) stenoteles. The last-fed hydranths, which showed the greatest decrease in stenotele activity, possessed 706 ± 45.1 (n = 3); thus, they had become inactivated with essentially a full complement of stenoteles. The hydranths which were fed second had 283 ± 40.9, while those which had been fed first retained only 98 ± 70.5 stenoteles (Table I).

Media exchange experiments

We tested the hypothesis that the accumulation of soluble factors in the feeding medium inactivated stenoteles of *H. disticha* by feeding starved hydranths in the media in which hydranths had previously been inactivated (Table II). The hydranths fed in "conditioned" media exhibited a greatly reduced killing response ($\bar{X}$ = 78.1% decrease; Table II). Interestingly, in a second experiment the control (original) hydranths killed more *Artemia* than did the controls of the first experiment, and the hydranths fed in this "used" solution were more inactivated ($\bar{X}$ = 94.5% decrease). This suggested a "dose-dependent" response, as if the inactivation were a function of the number of shrimp which were killed.

Volume dependency of inactivation

To investigate further the dose-dependent nature of stenotele inactivation during feeding, hydranths were tested in 150 ml of seawater, rather than the usual test volume of 0.15 ml. This would represent a thousand-fold dilution of any soluble factors.

TABLE II

Effects of feeding Halocordyle disticha *in seawater in which other hydranths had previously fed*

Experiment	Group	# of nauplii killed	% Decrease
1	Control	160	78.1%
	Experimental	35	
2	Control	261	95.4%
	Experimental	12	

The control group represents the first-fed group; experimental hydranths were fed in the solutions in which these had fed. Test solutions were filtered (0.45 μm) prior to use.
n = 2.

TABLE III

The effects of extracts of captitate tentacles and internodal tissue on stenotele function in Halocordyle disticha

Group	# of nauplii killed (±S.D.)
Fed in tentacle extract	0.0
Fed in internodal extract	192 ± 7.0
Controls	202 ± 12.0

Tissue extracts were prepared by electrifying tissue homogenates; controls were tested in electrified seawater.

Branches with three connected hydranths were selectively fed to inactivation. As in the previous experiments, the hydranth which was fed first killed the greatest number of *Artemia* ($\bar{X}$ = 237.5 ± 14.8; n = 2), the second-fed hydranth had an intermediate response ($\bar{X}$ = 117 ± 12.7; n = 2), and the third hydranth killed relatively few in comparison ($\bar{X}$ = 57.5 ± 7.8). The recovery of killing ability after transfer to fresh seawater was tested in these also. As in the connected hydranth experiment, the hydranth which had been fed first killed relatively few additional *Artemia* (20 ± 12.7; n = 2), the second-fed hydranth killed an intermediate number (54 ± 17; n = 2), and the hydranth fed third was relatively active (76 ± 8.5; n = 2). In all cases, the absolute numbers of shrimp killed by all three groups was somewhat higher than those which were tested in the smaller volume (see connected and separated experiments).

Feeding in prey homogenate

We examined the possibility that prey tissue might be the source of stenotele inactivating factors by feeding a branch with three hydranths in a solution containing the cell-free extract of 200 *Artemia* nauplii. This is approximately the number of *Artemia* killed by control hydranths throughout this study. Hydranths fed in electrified seawater alone killed 199.3 ± 4.0 nauplii (n = 4), while the experimental group killed on average 50 additional *Artemia* (249.3 ± 10.3; n = 4). This significant difference ($P < 0.01$; *t*-test) shows that prey homogenate stimulates the killing response. Thus inactivation cannot be due to factors in prey fluids which might be released during feeding.

The effects of feeding in a cell-free extract of capitate tentacles

An extract of capitate tentacles (see Materials and Methods) was used to determine if the tentacles were a source of inactivating factors. One control group was fed in electrified seawater, while a second control group was tested in an electrified extract of internodal tissue. While we had thought that the internodal tissue was nematocyst-poor, microscopic inspection of this tissue revealed that it contained many small nematocysts which could not be classified even with transmission electron microscopy (Clark, unpub.). Examination of electrified internodal tissue with light microscopy showed that none of these nematocysts were discharged. By contrast, 85–90% of the stenoteles of capitate tentacles were discharged by our electrification procedure.

Both control groups exhibited normal kill responses (Table III), indicating that neither metal-poisoning by platinum electrodes nor material from nematocyst-poor tissue had much effect on stenotele behavior. We observed no killing by any hydranths which were fed in the tentacle extract solution (n = 18; Table III). When the

inactivated hydranths from this experiment were transferred to fresh seawater, both control groups exhibited greater recovery ($\bar{X} = 43.3 \pm 5.3$ additional nauplii killed) than the experimental hydranths. These killed only 13 ± 5.3 nauplii, even though they had killed no prey during the experiment and presumably contained a full complement of stenoteles.

The effects of nematocyst discharge solution

The previous observations strongly suggested that stenoteles from capitate tentacles were the source of the inactivating factor. To directly test this possibility, we fed branches in a filtered solution of nematocyst discharge products (approximately 12,000 stenoteles in 0.15 ml), while controls were fed in electrified seawater. In three replicates, none of the experimental hydranths killed or captured any prey; controls exhibited a normal kill response ($\bar{X} = 183 \pm 19.7$ nauplii killed; $n = 12$). When the experimental hydranths were rinsed and placed in fresh seawater, they killed an average of four nauplii.

This was the lowest degree of stenotele recovery which we observed in any of our experiments. Both the complete inhibition of nematocyst activity and the low recovery represent exaggerated responses to the contents of approximately 20 times the number of stenoteles used during normal prey-killing (Table I).

The effects of hydroid or prey metabolites on the killing response

The waste products of hydroids or *Artemia* nauplii, produced under the conditions of our experiments, are possible sources of inactivating substances. We examined this possibility in two ways. In one experiment we kept branches with three hydranths in 0.15 ml of filtered seawater under microscope illumination for 1–3 h before feeding a single hydranth. All hydranths continued to kill nauplii, even after 3½h of these conditions. Each of the four hydranths tested killed at least 60 nauplii, and none were inactivated when feeding ended. In a second experiment, we kept a dense suspension of *Artemia* nauplii (*ca.* 220 nauplii per 0.15 ml) at room temperature for 1 h before the solution was filtered through bolting silk, and a single polyp on a three polyp branch selectively fed in 0.15 ml of the filtrate. In two trials inactivation was not achieved after 65 and 70 nauplii were killed; we did not present additional prey.

While these observations do not rule out inactivating effects of accumulated waste products such as CO_2, we feel that they are unlikely to be major factors for two reasons. First, our feeding experiments would be typically completed in less than an hour; second, the magnitude of inactivation we found in other experiments, particularly in third-fed hydranths and those treated with stenotele extracts, was much greater than what accumulated wastes could produce.

Discussion

Feeding responses and the kill response in H. disticha

Pardy and Lenhoff (1968) described prey killing in *Halocordyle disticha* (= *Pennaria tiarella*). Prey are immobilized by numerous small stenoteles of the filiform tentacles. This initiates the feeding responses which consist of the bending of the oral cone and the opening of the mouth. Then prey are actually killed by the large stenoteles of the capitate tentacles prior to engulfment of the prey by the mouth. "Feeding response" refers to the behavioral pattern which occurs after capture and before inges-

tion of prey. Pardy and Lenhoff (1968) demonstrated that this behavior is a response to free proline which is released in prey fluids following penetration of the body wall by stenoteles. While we often observed these responses, we were only concerned with the discharge of stenotele nematocysts (kill response) of *H. disticha* hydranths during feeding. Thus our findings on nematocyst inactivation do not contradict other studies (*e.g.*, Burnett *et al.*, 1968), which propose that nematocyst discharge in hydra may stimulate feeding responses.

Inactivation of nematocysts/cnidocytes during feeding in H. disticha

Our study shows that the prey-killing stenoteles of the colonial hydroid *H. disticha* are inactivated during periods of heavy feeding, although we cannot say if it is the responsiveness of cnidocyte or the nematocyst itself which is affected. Nematocyst inhibition following feeding has previously been reported in *Hydra* (Burnett *et al.*, 1960; Smith *et al.*, 1974; Ruch and Cook, 1984) and other cnidarians (Sandberg *et al.*, 1971; Mariscal, 1973). The reduction in nematocyst activity during feeding may be a general phenomenon among cnidarians which conserves these complex structures (Mariscal, 1974).

Previous workers had proposed that nematocyst inactivation during feeding by *Hydra* resulted either from the distention of endodermal stretch receptors following ingestion (Burnett *et al.*, 1960), or the reception of prey metabolites by gut cells (Smith *et al.*, 1974). Ruch and Cook (1984) showed that nematocyst/cnidocyte inhibition occurred in "gutless" hydra, without either of these sensory inputs. They ascribed inhibition to the accumulation of soluble factors released from a feeding hydra (Ruch and Cook, 1984). Our experiments involving media exchanges (Table II), the selective feeding of separated hydranths (Fig. 1), and recovery of stenotele activity are all consistent with the notion that this occurs in *H. disticha.* The effect is cumulative, being greater when more prey are killed (*cf.* the second- and third-fed hydranths in Fig. 1, and the media exchange experiments). Prey fluids are clearly not involved, as we found that prey extract stimulated stenotele discharge (*cf.* Ruch and Cook, 1984). Pardy and Lenhoff (1968) reported that proline (the "feeding activator" for *H. disticha,* doubtless present in prey fluids) elevated cnidocils on capitate tentacles of *H. disticha.* Their observation indicates increased responsiveness of cnidocytes following exposure to prey fluids. Our experiments also show that waste metabolites of the hydroids and the prey are unlikely to have a major role in inactivation.

Aside from ingestion and the presence of prey fluids, the most obvious difference between a feeding and non-feeding hydranth is the number of fired nematocysts. Our experiments provide evidence these fired nematocysts are a source of inactivating material. Both extracts of capitate tentacles and the discharge products of purified stenoteles produced complete inhibition. The results of the capitate tentacle extracts are particularly relevant, as these preparations contained more realistic concentrations of nematocyst discharge products (roughly those from 1–2 hydranths). Significantly, the extract of internodal tissue had no effect, even though it contained many small, and probably immature, nematocysts. These nematocysts were disrupted by our homogenization procedure, and the intracapsular material would have been present in the test solution. The implication is that discharge products, rather than intracapsular or non-nematocyst material, are responsible for inhibition. The preparation of purified stenoteles contained an unnaturally high concentration of discharge products, and the results of this experiment are an extreme case. Both of these experiments strongly suggest that mature, fired stenoteles are the source of inactivating substances, the most likely of which are the toxins themselves. The identity of these compounds

and how they are sensed are unknown, and we cannot say if they affect the cnidocyte or the nematocyst itself. Perhaps the cnidocil apparatus or other receptors on the ectodermal surface could be involved.

The "community" effect between hydranths in a colonial hydroid

Selective feeding of three hydranths revealed a community effect, in that feeding by one hydranth reduced the number of nauplii killed by neighboring hydranths in the same medium when they still contained functional stenoteles. Third-fed hydranths, which killed very few nauplii, were inactivated with virtually a full complement. This "community effect" is similar to the inactivation of attached buds due to parental feeding in hydra (Ruch and Cook, 1984) and of one head of a two-headed graft caused by the feeding of the other head (Smith *et al.*, 1974). As the products of ingestion by single polyps of colonial cnidarians can be transported to other areas of a colony (*H. disticha*, Rees *et al.*, 1970; gorgonians, Murdock, 1978; see also Gladfelter, 1983), inactivated hydranths would still receive the benefits of feeding by the original polyp. *H. disticha* is particularly interesting, given the possibility that non-feeding hydranths receive more from fed polyps than do feeding hydranths (Rees *et al.*, 1970).

Our results may reflect artifacts of feeding in small volumes and under static conditions. To what extent would such inactivation occur under natural conditions? Inactivation of stenoteles occurred both in 0.15 ml and 150 ml test volumes. The lack of an evident dilution effect suggests the chemical "halo" zone which Loomis (1961) postulated to exist around a hydra, or the "ultramicroenvironment" within 20 nm of the ectodermal surface where charged surface molecules could influence the concentration and binding of molecules to the surface (Lenhoff, 1965). Ruch and Cook (1984) found similar effects of dilution on nematocyst inactivation in *Hydra*. Possibly the boundary layers which exist around polyps when exposed to flow situations in nature (see Patterson, 1984, and references therein) would also permit the accumulation of inactivating factors in the vicinity of a heavily feeding hydranth. Whether "neighborhood inactivation" actually occurs in the field, particularly in tidal flow situations where *H. disticha* commonly occurs, is not clear. Experiments involving controlled feeding of individual hydranths of *H. disticha* would be difficult under field conditions of tidal flow.

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